

# $^1\text{H}$ Nuclear Magnetic Resonance Studies of the Conformations of Adrenocorticotropin Hormone ACTH(1—10) and Related Peptides in Aqueous and Trifluoroethanol Solutions

Brian J. Rawson, James Feeney,\* and Barry J. Kimber  
National Institute for Medical Research, Mill Hill, London NW7 1AA  
H. M. Greven  
Organon Scientific Development Group, Oss, Holland

We have measured the 270 MHz  $^1\text{H}$  n.m.r. spectra of adrenocorticotropin hormone ACTH(1—10) and related peptides in aqueous and trifluoroethanol solution at a series of temperatures. In aqueous solution the  $J_{\text{NC}}$  coupling constants (between  $\alpha\text{-CH}$  and NH protons) and the temperature dependence of the peptide NH proton chemical shifts indicate that there is a random coil mixture of conformations. Shielding interactions between the side-chains of the aromatic residues L-Phe-7 and L-Trp-9 are observed and shown to be expected from a random coil mixture of conformations where the side-chains of alternate residues are close together in some conformations. In trifluoroethanol solutions, the data indicate that the overall conformation of ACTH(1—10) is displaced somewhat from the random coil mixture: while the  $J_{\text{NC}}$  coupling constants indicate that there is not a dominant contribution from helical conformations, some contribution from these forms is suggested by the presence of some intramolecular hydrogen bonding involving peptide NH protons.

Adrenocorticotropin hormone (ACTH) has several endocrine functions (for example, the stimulation of corticosteroid production in the adrenal cortex) and in addition is known to influence certain behavioural responses.<sup>1-3</sup>

It has been established that the 1—24 fragment of the 39-residue polypeptide hormone retains the full endocrine activity.<sup>1</sup> This activity is reduced in smaller fragments but several of these such as ACTH(4—9) and ACTH(1—10) (A) have been shown<sup>3</sup> to have an activity equal to that of ACTH itself in their ability to influence such behavioural responses as conditioned active and passive avoidance behaviour in rats. Studies on the relationship between structure and behavioural activity suggest that ACTH(4—10) has a helical-like conformation at the receptor site.<sup>2</sup>

Several workers<sup>3-5</sup> have pointed out that the sequence Met-Glu-His-Phe-Arg-Trp (residues 4—9 of ACTH) possesses considerable potential for the formation of helical structures.<sup>6</sup> It is of interest to examine the extent to which this potential is realised by ACTH fragments of different length and to study the effect of solvent on any helicity found.

Spectroscopic evidence involving c.d.<sup>7</sup> and  $^{13}\text{C}$  n.m.r.<sup>8</sup> have strongly suggested that ACTH and its 1—24 fragment readily adopt a helical conformation in trifluoroethanol. Toma and his co-workers<sup>8</sup> have suggested on the basis of their work with  $^{13}\text{C}$  n.m.r. that there may be helical conformations present even in aqueous solution and specifically in the region of the 4—9 residues. Patel<sup>9</sup> has found from  $^1\text{H}$  n.m.r. studies that there are no interactions between sequences 1—10 and 11—24 in ACTH(1—24).

In this paper we report the results of a  $^1\text{H}$  n.m.r. study of the conformations of (A) and related fragments in aqueous and trifluoroethanol solutions. We have included in this study peptides containing D-Phe at position 7 in place of the naturally occurring L-Phe because these peptides show effects opposite to those of the corresponding L-peptides when tested on extinction of an active avoidance response in rats.<sup>2</sup>

## Experimental

The peptides examined were ACTH(1—10) (A), ACTH(4—10), ACTH(7—10), and the corresponding D-Phe-7 analogues. They were prepared by the classical method of fragment

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly

(A)

condensation using the approach of Schwyzer and Kappeler.<sup>10</sup> Their  $^1\text{H}$  n.m.r. spectra were as expected for the various peptides and indicated the absence of any impurities present in significant amounts.

Where possible, concentrations of 3—5 mM were used but in aqueous solution at pH 7 limited solubility necessitated using lower concentrations, particularly in the case of the peptides containing L-Phe isomer. ACTH(1—10) was particularly difficult to dissolve at pH 7 and adequate spectra were obtained only with difficulty. The spectra observed were not sensitive to change in concentration.

The  $^1\text{H}$  n.m.r. spectra were recorded using a 90° pulse angle at 270 MHz with a Bruker WH270 spectrometer operating in the Fourier transform mode. Depending on the concentration of the sample, between 400 and 2000 f.i.d.s were accumulated in 8 K data points using a spectral width of 4200 Hz. Before Fourier transformation the f.i.d. was multiplied by an exponentially decreasing function which improved the sensitivity of the transformed spectrum and gave a linewidth contribution of 0.2 Hz.

Chemical shifts were measured relative to tetramethylsilane (TMS) in trifluoroethanol solutions and relative to 1,4-dioxan in aqueous solutions [and quoted relative to 4,4-dimethyl-4-silapentanesulphonate (DSS) by the addition of 3.71 p.p.m.]. Neutral aqueous solutions were buffered in 50 mM-potassium phosphate whilst acid solutions were pH adjusted with 1M- $^2\text{HCl}$ . pH Measurements were made using a Radiometer model 26 pH meter equipped with a glass electrode. No attempt was made to correct for deuterium isotope effects on pH values measured in  $\text{D}_2\text{O}$  solution.

In order to examine the peptides in either  $\text{H}_2\text{O}$  or trifluoroethanol solution it was necessary to overcome the dynamic range problem posed by the intense solvent signals. For the aqueous solutions, providing that concentrations of ca. 3 mM were available this was achieved by pre-irradiating at the water resonance frequency for 0.5 s to saturate the water protons prior to applying the radiofrequency pulse and acquiring data. For more dilute aqueous samples and for the solutions in trifluoroethanol the data were accumulated using

an increased word length in the computer (double word-length format) and carrying out the Fourier transformation using floating point arithmetic.<sup>11</sup> For H<sub>2</sub>O and trifluoroethanol solutions the instrument was locked onto 10% <sup>2</sup>H<sub>2</sub>O and trifluoroethan[<sup>2</sup>H]ol added to the non-deuteriated solvents.

<sup>2</sup>H<sub>2</sub>O (99.85% <sup>2</sup>H) was obtained from Norsk Hydroelektrisk and trifluoroethanol from Sigma Chemicals. Trifluoroethanol was used without further purification and probably contained traces of water.

## Results and Discussion

<sup>1</sup>H Spectra in <sup>2</sup>H<sub>2</sub>O Solutions.—The spectrum of each of the six peptides was recorded under neutral (pH 7.0) and acid (pH 2.0) conditions. The results for the non-exchangeable protons are given in Table 1.

The fact that the peptides constituted two series with increasing chain lengths considerably simplified the problem of assignment of resonances in these spectra. Comparisons within these series, correlation with published data from model peptides,<sup>12</sup> and application of spin-decoupling experiments allow the assignments given in Table 1 to be made.

The resonances of the β-CH<sub>2</sub> protons of the aromatic residues, Tyr-2, His-6, and Phe-7 considerably overlap with each other and with the δ-CH<sub>2</sub> resonance from Arg-8. Although the α-CH resonances connected to these β-CH<sub>2</sub> multiplets were usually not directly observable, being obscured by the residual HDO peak, their position could be determined to an accuracy of ±0.015 p.p.m. by irradiation under the HDO peak and observing the collapse of the β-signals. Thus each β-signal could readily be connected with its corresponding α-resonance, and it remained to assign these to the individual residues. The signal from the α-CH of tyrosine was assigned by its coupling with the very low field NH proton in the <sup>1</sup>H<sub>2</sub>O spectra. For the D-Phe containing peptides it was not possible to make unequivocal assignments for the Phe-7 and His-6 α- and β-protons because of overlap of the β-CH<sub>2</sub> signals.

It is apparent from the data given in Table 1 that extension of the chain from 7—10 through 4—10 to 1—10 causes little change in the shifts of the resonances of the common protons except for variations expected when a residue is changed from being at the nitrogen terminus to being elsewhere in the chain. This indicates that in aqueous solution extension of the chain does not bring about any dramatic changes in peptide conformation.

In all the L-Phe ACTH fragments, the Phe aromatic absorptions appear as two distinct multiplets resonating at δ 7.02 and 7.20 while the D-Phe ACTH fragments give resonances in the region δ 7.12—7.34. This can be seen clearly in the aromatic regions of the <sup>1</sup>H n.m.r. spectra of the L-Phe and D-Phe ACTH(1—10) shown in Figure 1. In small peptides containing a Phe residue as the only aromatic amino-acid, the aromatic protons normally resonate at δ 7.30—7.40.

<sup>1</sup>H Spectra in <sup>1</sup>H<sub>2</sub>O Solution.—Using the solvent suppression technique described above, spectra were measured as a function of temperature for each of the peptides under acidic conditions (pH 2.0). Under neutral conditions well resolved spectra for the NH protons were unobtainable because of broadening due to exchange with solvent protons. Table 2 summarises the NH <sup>1</sup>H chemical shifts, *J*<sub>NH</sub> coupling constants (between the α-CH and the NH protons), and the temperature dependence of the NH chemical shifts for each residue. The assignments were made by decoupling of the NH multiplets on irradiation of the corresponding α signals. No ambiguity exists except where α-signals overlap or where, as

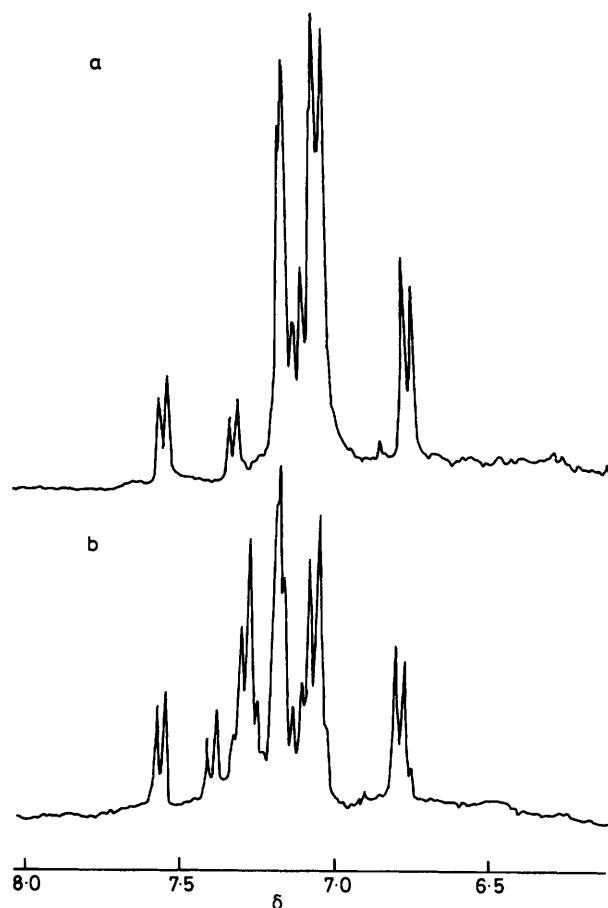


Figure 1. The aromatic regions of the <sup>1</sup>H 270 MHz n.m.r. spectra of ACTH(1—10) (a) and D-Phe-7 ACTH(1—10) (b) examined in D<sub>2</sub>O solution (pH 2) at 35 °C

in the case of the Phe and His residues of the D-Phe peptides the β-signals themselves are not unambiguously assigned. For the peptide fragments examined here the measured values of the *J*<sub>NH</sub> coupling constants were all fairly large (5.8—8.5 Hz).

<sup>1</sup>H Spectra in Trifluoroethanol Solution.—The L-Phe ACTH(1—10) and D-Phe ACTH(1—10) peptides were also examined in trifluoroethanol solution at 30, 35, and 40 °C. Figure 2a shows the low field region of the <sup>1</sup>H spectrum of L-Phe ACTH(1—10) in this solvent and it is seen that several well resolved NH proton signals (labelled A—E) were observed in addition to the aromatic protons. The NH chemical shifts, *J*<sub>NH</sub> spin coupling constants and the temperature coefficients of the NH chemical shifts are given in Table 3. A detailed assignment of these protons has not been made but it is clear that the *J*<sub>NH</sub> coupling constant and the temperature coefficients are somewhat lower than the range of values measured in H<sub>2</sub>O solution.

The chemical shifts of the aromatic protons in both the L-Phe and D-Phe isomers are given in Table 4: the Trp, Phe, and Tyr signals have very similar chemical shifts in the two isomers. This can be seen by comparing the spectra of the aromatic protons in Figures 2a and b.

These results contrast with those observed in aqueous solutions where the Phe protons have quite different <sup>1</sup>H shifts in the spectra of the two peptides (see Figure 1).

*Backbone Conformation.*—Gibbons and his co-workers<sup>13</sup>

**Table 1.** The  $^1\text{H}$  chemical shifts  $\dagger$  and assignments for ACTH(1–10) and related peptides at 35 °C in aqueous solution ( $^2\text{H}_2\text{O}$ )

| Residue                                   | Peptides with L-Phe-7 |      |      |       |           |      | Peptides with D-Phe-7 |           |      |      |           |           |
|---|-----------------------|------|------|-------|-----------|------|-----------------------|-----------|------|------|-----------|-----------|
|   | 4–10                  |      | 7–10 |       | 1–10      |      | 4–10                  |           | 7–10 |      | 1–10      |           |
|   | pH 7                  | pH 2 | pH 7 | pH 2  | pH 7      | pH 2 | pH 7                  | pH 2      | pH 7 | pH 2 | pH 7      | pH 2      |
| Gly-10 $\text{C}_\alpha\text{H}_\text{A}$ | 3.71                  | 3.92 | 3.71 | 3.91  | {3.87}    |      | 3.71                  | {3.90}    | 3.76 | 3.90 | {3.88}    |           |
| $\text{C}_\alpha\text{H}_\text{B}$        | 3.63                  | 3.89 | 3.61 | 3.89  |           |      | 3.63                  |           | 3.57 | 3.83 |           |           |
| $J_{\text{gem}}$                          | 16.9                  | 18.4 | 17.3 | 18.35 |           |      | 17.2                  |           | 17.5 | 18.1 |           |           |
| Trp-9 C(2)H                               | 7.22                  | 7.22 | 7.24 | 7.26  | 7.22      | 7.21 | 7.19                  |           | 7.20 | 7.21 | 7.18      |           |
| C(4)H                                     | 7.63                  | 7.61 | 7.66 | 7.66  | 7.62      | 7.61 | 7.61                  | 7.59      | 7.61 | 7.61 | 7.63      | 7.58      |
| C(5)H                                     | 7.13                  | 7.16 | 7.15 | 7.15  | 7.11      | 7.14 | 7.10                  | 7.12      | 7.12 | 7.12 | 7.08      | 7.08      |
| C(6)H                                     | 7.15                  | 7.16 | 7.16 | 7.16  | 7.17      | 7.19 | 7.12                  | 7.15      | 7.16 | 7.15 | 7.13      | 7.16      |
| C(7)H                                     | 7.39                  | 7.32 | 7.39 | 7.34  | 7.39      | 7.37 | 7.39                  | 7.42      | 7.44 | 7.45 | 7.38      | 7.41      |
| $\text{C}_\alpha\text{H}$                 | 4.68                  | 4.68 | 4.64 | 4.65  | 4.67      |      | 4.73                  | 4.70      | 4.74 | 4.70 | 4.69      |           |
| $\text{C}_\beta\text{H}_\text{A}$         | 3.15                  | 3.18 | 3.17 | 3.20  | 3.17      |      | 3.15                  | 3.16      | 3.13 | 3.17 | 3.15      | 3.15      |
| $\text{C}_\beta\text{H}_\text{B}$         | 3.32                  | 3.30 | 3.33 | 3.32  | 3.30      |      | 3.33                  | 3.30      | 3.35 | 3.30 | 3.31      | 3.29      |
| Arg-8 $\text{C}_\alpha\text{H}$           | 4.18                  | 4.16 | 4.20 | 4.24  | 4.17      |      | 4.08                  | 4.07      | 3.85 | 3.94 | 4.07      |           |
| $\text{C}_\beta\text{H}$                  | 1.52                  | 1.59 | 1.53 | 1.58  | 1.57      |      | 1.40                  | 1.40      | 1.11 | 1.19 | 1.41      | 1.41      |
|   |                       |      |      |       |           |      | 1.31                  | 1.24      |      |      | 1.33      | 1.27      |
| $\text{C}_\gamma\text{H}$                 | 1.32                  | 1.36 | 1.32 | 1.41  | 1.34      |      | 1.05                  | 0.94      | 0.80 | 0.84 | 1.07      | 0.96      |
| $\text{C}_\delta\text{H}$                 | 3.02                  | 3.05 | 3.01 | 3.05  | 3.05      |      | 2.90                  | 2.88      | 2.79 | 2.83 | 2.88      |           |
| Phe-7 Aromatics *                         | 7.17                  | 7.20 | 7.17 | 7.09  | 7.16      | 7.20 | 7.26                  | 7.29      | 7.30 | 7.34 | 7.25      | 7.28      |
|   | 7.05                  | 7.10 | 7.02 | 7.02  | 7.08      | 7.09 | 7.12                  | 7.19      | 7.20 | 7.23 | 7.14      | 7.18      |
| $\text{C}_\alpha\text{H}$                 | 4.43                  | 4.50 | 3.89 | 4.12  | 4.47      |      | 4.51                  | 4.52      | 3.88 | 4.12 | 4.48      | 4.47      |
|   |                       |      |      |       | (or 4.54) |      | (or 4.48)             | (or 4.66) |      |      |           | (or 4.62) |
| $\text{C}_\beta\text{H}$                  | 2.84                  | 2.79 | 2.89 | 2.94  | 2.81      |      | 2.91                  | 2.95      | 3.12 | 3.22 | 4.62      |           |
|   |                       |      | 2.86 | 2.86  | (or 3.06) |      | (or 2.88)             | (or 3.01) | 2.91 | 3.03 |           |           |
|   |                       |      |      |       | 3.01      |      | 2.81                  | 2.95      |      |      |           |           |
| His-6 C(2)H                               | 7.84                  | 8.53 |      |       | 7.80      | 8.49 | 7.88                  | 8.52      |      |      | 7.84      | 8.41      |
| C(4)H                                     | 6.92                  | 7.11 |      |       | 6.80      | 7.11 | 6.86                  | 7.09      |      |      | 6.82      | 7.04      |
| $\text{C}_\alpha\text{H}$                 | 4.47                  | 4.55 |      |       | 4.54      |      | 4.51                  | 4.56      |      |      | 4.62      |           |
|   |                       |      |      |       | (or 4.47) |      | (or 4.48)             | (or 4.52) |      |      | (or 4.47) |           |
| $\text{C}_\beta\text{H}$                  | 2.95                  | 3.10 |      |       | 3.06      |      | 2.91                  | 3.10      |      |      | 4.18      |           |
|   |                       | 2.99 |      |       | 3.01      |      | (or 2.81)             | 2.98      |      |      | 1.81      |           |
|   |                       |      |      |       | (or 2.81) |      | (or 2.88)             | (or 2.95) |      |      | 2.25      |           |
| Glu-5 $\text{C}_\alpha\text{H}$           | 4.23                  | 4.27 |      |       | 4.17      |      | 4.24                  | 4.28      |      |      | 4.18      |           |
| $\text{C}_\beta\text{H}$                  | 1.82                  | 1.85 |      |       | 1.84      |      | 1.83                  | 1.85      |      |      | 1.77      | 1.81      |
| $\text{C}_\gamma\text{H}$                 | 2.12                  | 2.30 |      |       | 2.30      |      | 2.13                  | 2.29      |      |      | 2.25      |           |
| Met-4 $\text{C}_\alpha\text{H}$           | 3.95                  | 4.09 |      |       | 4.37      |      | 3.90                  | 4.07      |      |      | 4.37      |           |
| $\text{C}_\beta\text{H}$                  | 1.98                  | 2.09 |      |       | 2.02      |      | 1.99                  | 2.06      |      |      | 1.94      |           |
| $\text{C}_\gamma\text{H}$                 | 2.46                  | 2.47 |      |       | 2.50      |      | 2.45                  | 2.48      |      |      | 2.46      | 2.46      |
| $\text{C}_\delta\text{H}$                 | 1.96                  | 2.05 |      |       | 2.04      |      | 2.00                  | 2.03      |      |      | 2.00      | 2.02      |
| Ser-3 $\text{C}_\alpha\text{H}$           |                       |      |      |       | 4.35      |      |                       |           |      |      | 4.35      |           |
| $\text{C}_\beta\text{H}$                  |                       |      |      |       | 3.76      |      |                       |           |      |      | 3.75      |           |
| Tyr-2 $\text{C}_\alpha\text{H}$           |                       |      |      |       | 4.62      |      |                       |           |      |      | 4.62      |           |
| $\text{C}_\beta\text{H}$                  |                       |      |      |       | 2.96      |      |                       |           |      |      | 2.97      |           |
| C(3)(5)                                   |                       |      |      |       | 6.77      | 6.78 |                       |           |      |      | 6.76      | 6.77      |
| C(2)(6)                                   |                       |      |      |       | 7.06      | 7.07 |                       |           |      |      | 7.04      | 7.07      |
| Ser-1 $\text{C}_\alpha\text{H}$           |                       |      |      |       | 4.08      |      |                       |           |      |      | 4.07      |           |
| $\text{C}_\beta\text{H}$                  |                       |      |      |       | 3.93      |      |                       |           |      |      | 3.93      |           |

\* Centre of multiplet.  $\dagger$  Chemical shifts accurate to  $\pm 0.015$  p.p.m.

**Table 2.** The  $J_{\text{NC}}$  coupling constants (Hz),  $^1\text{H}$  chemical shifts, and their temperature coefficients ( $\times 10^3$  p.p.m.  $\text{K}^{-1}$ ) for the NH protons in ACTH(1–10) and related peptides in  $^2\text{H}_2\text{O}$  solution

|                             | Peptide with L-Phe-7 |                         | Peptide with D-Phe-7 |             |               |
|-----------------------------|----------------------|-------------------------|----------------------|-------------|---------------|
|                             | 4–10                 | 1–10                    | 4–10                 | 7–10        | 1–10          |
| Gly-10 $\delta_{\text{NH}}$ | 8.10                 | 8.05                    | 8.06                 | 8.06        | 8.00          |
| $J_{\text{NC}}$             | $6 \pm 0.5$          | $5.5 \pm 0.5$           | $5.5 \pm 0.5$        | $6 \pm 0.5$ | $6 \pm 0.5$   |
| $\Delta\delta/\Delta T$     | 0.006                | 0.006                   | 0.006                |             | 0.006         |
| Trp-9 $\delta_{\text{NH}}$  | 7.86                 | 7.82                    | 7.98                 | 8.08        | 7.96          |
| $J_{\text{NC}}$             | $7.5 \pm 0.5$        | $7.3 \pm 0.5$           | $7.5 \pm 0.5$        | $8 \pm 0.5$ |               |
| $\Delta\delta/\Delta T$     | 0.006                | 0.007                   | 0.006                |             | 0.006         |
| Arg-8 $\delta_{\text{NH}}$  | 8.16                 | 7.99                    | 8.02                 | 8.06        | 7.97          |
| $J_{\text{NC}}$             | $7.5 \pm 0.5$        | 7 ± 0.5<br>(or<br>8.05) | $8 \pm 0.5$          | $6 \pm 0.5$ | $6 \pm 0.5$   |
| $\Delta\delta/\Delta T$     | 0.006                | 0.006<br>(or<br>0.005)  | 0.008                |             | 0.006         |
| Phe-7 $\delta_{\text{NH}}$  | 8.18                 | 8.00                    | 8.31                 |             | 8.21          |
| $J_{\text{NC}}$             | $7 \pm 0.5$          | $6 \pm 1$               | $6 \pm 0.5$          |             | $8 \pm 0.5$   |
| $\Delta\delta/\Delta T$     | 0.008                | 0.007                   | 0.008                |             | 0.006         |
| His-6 NH                    | 8.48                 | 8.23                    | 8.49                 |             | 8.24          |
| $J_{\text{NC}}$             | $7.5 \pm 0.5$        | $8.5 \pm 1$             | $7 \pm 0.5$          |             | $8 \pm 0.5$   |
| $\Delta\delta/\Delta T$     | 0.007                | 0.006                   | 0.006                |             | 0.006         |
| Glu-5 $\delta_{\text{NH}}$  | 8.63                 | 8.05                    | 8.59                 |             | 8.02          |
| $J_{\text{NC}}$             | $6 \pm 0.5$          | 8 ± 0.5<br>(or<br>7.99) | $6 \pm 0.5$          |             | $6.5 \pm 0.5$ |
| $\Delta\delta/\Delta T$     | 0.006                | 0.005<br>(or<br>0.006)  | 0.006                |             | 0.005         |
| Met-4 $\delta_{\text{NH}}$  |                      | 8.18                    |                      |             | 8.18          |
| $J_{\text{NC}}$             |                      | $6.5 \pm 0.5$           |                      |             | $8 \pm 0.5$   |
| $\Delta\delta/\Delta T$     |                      | 0.007                   |                      |             | 0.007         |
| Ser-3 $\delta_{\text{NH}}$  |                      | 8.18                    |                      |             | 8.18          |
| $J_{\text{NC}}$             |                      | $6.5 \pm 0.5$           |                      |             | $8 \pm 0.5$   |
| $\Delta\delta/\Delta T$     |                      | 0.007                   |                      |             | 0.007         |
| Tyr-2 $\delta_{\text{NH}}$  |                      | 8.56                    |                      |             | 8.54          |
| $J_{\text{NC}}$             |                      | $7 \pm 0.5$             |                      |             | $6.5 \pm 0.5$ |
| $\Delta\delta/\Delta T$     |                      | 0.006                   |                      |             | 0.006         |

**Table 3.**  $^1\text{H}$  Chemical shifts of the NH protons in ACTH(1–10) in trifluoroethanol at 35 °C

| NH proton     | $^1\text{H}$ Chemical shift ( $\delta$ ) | $J_{\text{NC}}$ coupling constant (Hz) | $10^{-3}\Delta\delta/\Delta T$ (p.p.m. $\text{K}^{-1}$ ) |
|---------------|--|--|--|
| A             | 7.47                                     | 6.5                                    |  |
| B             | 7.50                                     | 7.6                                    | 7.8  |
| C             | 7.67                                     | 6.3                                    | 4.4  |
| D             | 7.76                                     | $5.5 \pm 0.5$                          | 3.7  |
| E             | 7.84                                     | 5                                      | 6  |
| F             | 8.65                                     | Broad *                                | (3.1) *  |
| G<br>(indole) | 9.17                                     |  | 5  |

\* This NH is almost certainly from the penultimate Tyr residue and is influenced by exchange with the solvent: this will influence both the linewidth and the  $\Delta\delta/\Delta T$  value.

and other workers<sup>14–16</sup> have shown how backbone conformational information can be obtained from the  $J_{\text{NC}}$  coupling constants measured from the multiplet splittings on the NH signals. The  $J_{\text{NC}}$  coupling constants have been related to the  $\theta$  dihedral angle in the  $\alpha\text{-CH-NH}$  fragment by the Bystrov-Karplus<sup>16</sup> equation (1). It has been shown that a mixture of

$$J_{\text{NC}} = 8.9 \cos^2(300 + \varphi) - 0.9 \cos(300 + \varphi) + 0.9 \sin^2(300 + \varphi) \quad (1)$$

conformations with  $\varphi$  angles populated according to a random coil distribution would give  $J_{\text{NC}}$  values of  $7 \pm 1$  Hz.<sup>13</sup> These high values are consistent with a distribution of conformations with  $\varphi, \psi$  angles as described by potential energy maps of the type calculated by Sheraga and his co-workers<sup>17,18</sup> for *N*-acetyl-*N'*-methylamides of amino-acids<sup>17</sup> and simple dipeptides.<sup>18</sup>

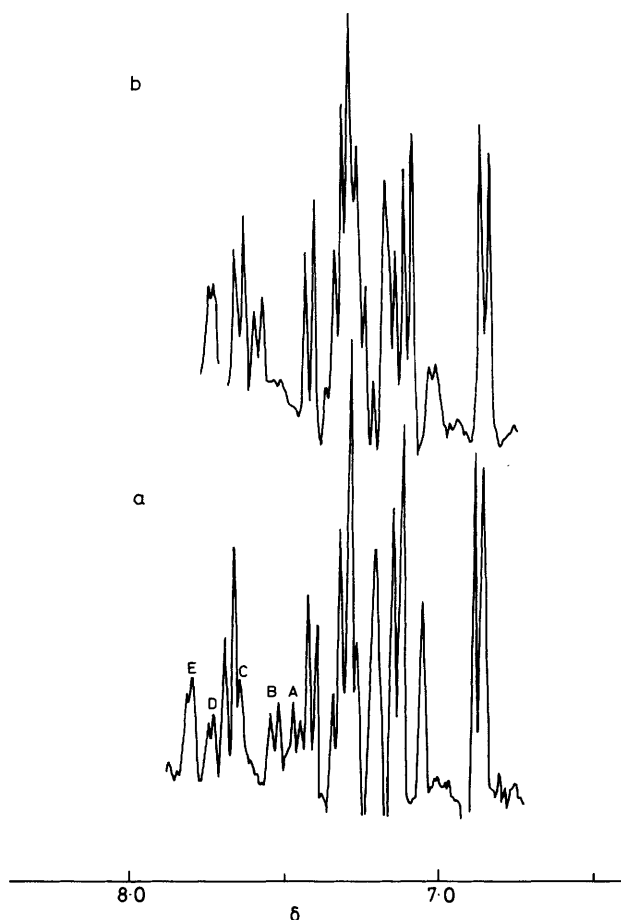
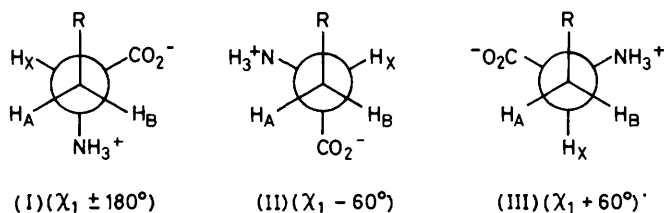


Figure 2. The aromatic region of the convolution difference  $^1\text{H}$  270 MHz n.m.r. spectra of ACTH(1—10) (a) and D-Phe-7 ACTH(1—10) (b) in trifluoroethanol at 35 °C



The  $\phi$  angle for a residue in an  $\alpha$ -helix ( $\phi \pm 47^\circ$ ) would lead to  $J_{\text{NC}}$  values of  $< 3$  Hz. Clearly the large  $J_{\text{NC}}$  values (5.8—8.5 Hz) measured for the ACTH peptide fragments in  $\text{H}_2\text{O}$  solution are consistent with a random coil distribution and indicate that there is not a dominant contribution from  $\alpha$ -helical structures. The  $J_{\text{NC}}$  values measured in trifluoroethanol solution are significantly lower than the values in  $\text{H}_2\text{O}$  solution (see Tables 2 and 3) but none of them is sufficiently small to indicate a single dominant helical conformation.

The temperature dependence of the NH proton chemical shifts in aqueous solution provides further evidence for the absence of a large contribution from  $\alpha$ -helical structures. For all the peptide bond NH protons the temperature coefficients are large [ $(5-7) \times 10^{-3}$  p.p.m.  $\text{K}^{-1}$ ] and similar to those linear peptides in which no intramolecular hydrogen bonding is present.<sup>13,19,20</sup> The observed changes in shift with temperature are due to changes in the extent of hydrogen bonding of the

Table 4.  $^1\text{H}$  Chemical shifts of the aromatic protons in ACTH(1—10) and D-Phe ACTH(1—10) in trifluoroethanol at 35 °C

| Residue         | Chemical shifts ( $\delta$ ) |             |
|-----------------|------------------------------|-------------|
|                 | L-Phe(1—10)                  | D-Phe(1—10) |
| Trp 4-H         | 7.70                         | 7.68        |
| Trp 7-H         | 7.41                         | 7.44        |
| Trp 2-, 5-, 6-H | {7.29}                       | {7.32}      |
| Phe 1—5-H       | {7.20}                       | {7.20}      |
| Tyr 2-, 6-H     | 7.13                         | 7.11        |
| Tyr 3-, 5-H     | 6.85                         | 6.85        |

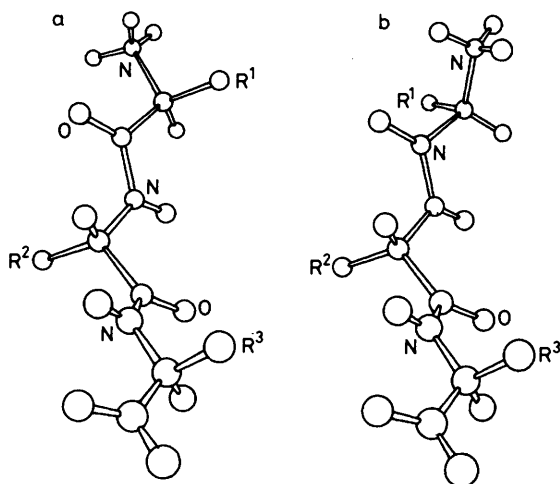
NH protons with the solvent as the temperature is changed: when the NH proton is involved in an intramolecular hydrogen bond the temperature coefficient of the NH proton chemical shifts are much lower ( $3 \times 10^{-3}$  p.p.m.  $\text{K}^{-1}$ ).<sup>13,19,20</sup> Since  $\alpha$ -helical structures are stabilised by intramolecular hydrogen bonds the absence of any strong contribution from such hydrogen bonding to the observed temperature coefficients of the NH  $^1\text{H}$  shifts again indicates that there is not a dominant contribution from helical structures to the overall population of conformers in these peptides in aqueous solution. The temperature coefficients of the NH proton chemical shifts in trifluoroethanol show a much larger variation ( $\Delta\delta/\Delta T$  values in the range  $3.7-7.8 \times 10^{-3}$  p.p.m.  $\text{K}^{-1}$ ) than those in aqueous solution. Two of the NH protons have particularly low values ( $\Delta\delta/\Delta T$  3.7 and  $4.4 \times 10^{-3}$  p.p.m.  $\text{K}^{-1}$ ) suggesting that these are involved in intramolecular hydrogen bonding in some of their conformations.

*Side-chain Conformations.*—For several residues it was possible to resolve the  $\alpha\text{-CH}-\beta\text{-CH}_2$  multiplets in the spectra of the peptides and to extract the two three-bond coupling constants ( $J_{\alpha\beta_A}$  and  $J_{\alpha\beta_B}$ ) from an ABX analysis of the multiplets. These coupling constants can be used to estimate the fractional populations  $P_I$ ,  $P_{II}$ , and  $P_{III}$  of rotamers (I)—(III) for side-chains in  $\alpha$ -amino-acids. The two measured vicinal coupling constants are taken as averaged values of their component coupling constants in the rotamers weighted according to their fractional populations. The values of the component coupling constants [ $J_g$  (*gauche*) and  $J_t$  (*trans*)] on the rotamers have been estimated by several workers.<sup>21-23</sup> Using Pachler's<sup>21</sup> values of  $J_g$  2.56 and  $J_t$  13.6 Hz and making the approximation that they are the same in the different rotamers, it is possible to estimate the fractional populations of the side-chains from the measured values of  $J_{\alpha\beta_A}$  and  $J_{\alpha\beta_B}$ . The values of these coupling constants for the Phe and Trp residues in the ACTH peptides in aqueous solution are given in Table 5, together with the calculated rotamer populations. While the absolute values of these rotamer populations will have errors of *ca.*  $\pm 0.1$  the method is useful for detecting the most populated conformation and for monitoring changes in side-chain populations in the different peptides. It should be mentioned that unless the  $\beta\text{-CH}_2$  signals can be assigned unambiguously to  $\text{H}_A$  and  $\text{H}_B$  in the rotamers then there is an ambiguity in assigning rotamers (I) and (II): we have assumed that the assignments of  $\text{H}_A$  and  $\text{H}_B$  in these peptides are the same as found in model peptides.<sup>17,24,25</sup> None of the changes in the  $\beta\text{-CH}_2$   $^1\text{H}$  shifts are sufficiently different from those in model peptides to suggest that the assignments might be reversed. The only significant difference seen in the side chain populations of the Phe and Trp residues is in the ACTH(7—10) series where the Phe is at the nitrogen terminal position. Otherwise there is no evidence for any differences in side-chain interactions in the various peptides.

**Table 5.** The  $J_{\alpha\beta}$  coupling constants and rotamer populations in the Phe and Trp side-chains of ACTH(1–10) and related peptides

|                     | Peptides with L-Phe-7 |      |      |      |      | Peptides with D-Phe-7 |      |      |      |      |   |
|---------------------|-----------------------|------|------|------|------|-----------------------|------|------|------|------|---|
|                     | 4–10                  |      | 7–10 |      | 1–10 | 4–10                  |      | 7–10 |      | 1–10 |   |
|                     | pH 7                  | pH 2 | pH 7 | pH 2 | pH 2 | pH 7                  | pH 2 | pH 7 | pH 2 | pH 2 |   |
| <b>Phe-7</b>        |                       |      |      |      |      |                       |      |      |      |      |   |
| $J_{\alpha\beta_A}$ | {7.7}                 |      | *    | 7.7  | 7.7  | 7.4                   | *    | *    | 9.85 | 10.1 | * |
| $J_{\alpha\beta_B}$ | {7.7}                 |      | *    | 6.7  | 7.5  | 7.4                   | *    | *    | 5.55 | 5.5  | * |
| $J_{\beta\beta}$    |                       |      | 13.6 | 14.1 | —    |                       |      | 13.2 | 13.3 |      |   |
| $P_I$               | 0.46                  |      | 0.40 | 0.45 | 0.44 |                       |      | 0.27 | 0.27 |      |   |
| $P_{II}$            | 0.46                  |      | 0.46 | 0.46 | 0.44 |                       |      | 0.66 | 0.68 |      |   |
| $P_{III}$           | 0.08                  |      | 0.14 | 0.09 | 0.12 |                       |      | 0.07 | 0.05 |      |   |
| <b>Trp-9</b>        |                       |      |      |      |      |                       |      |      |      |      |   |
| $J_{\alpha\beta_A}$ | 8.8                   | 8.0  | 8.5  | 8.0  | 8.2  | 8.8                   | 8.5  | 9.8  | 8.5  | 8.7  |   |
| $J_{\alpha\beta_B}$ | 5.7                   | 5.8  | 5.7  | 6.3  | 5.9  | 5.4                   | 5.7  | 5.9  | 7.0  | 5.9  |   |
| $J_{\beta\beta}$    | 14.7                  | 14.6 | 14.7 | 15.1 | 14.8 | 14.7                  | 14.5 | 14.9 | 14.6 | 14.7 |   |
| $P_I$               | 0.28                  | 0.29 | 0.28 | 0.34 | 0.30 | 0.26                  | 0.28 | 0.30 | 0.40 | 0.30 |   |
| $P_{II}$            | 0.56                  | 0.49 | 0.54 | 0.49 | 0.51 | 0.56                  | 0.54 | 0.65 | 0.54 | 0.56 |   |
| $P_{III}$           | 0.16                  | 0.22 | 0.18 | 0.17 | 0.19 | 0.18                  | 0.18 | 0.05 | 0.06 | 0.14 |   |

\* The  $\beta\text{CH}_2$  multiplets for these residues could not be analysed unequivocally because of overlap with other  $\beta\text{-CH}$  signals. Errors in rotamer populations  $\pm 0.1$ . Errors in  $J_{\alpha\beta}$  values  $\pm 0.25$  Hz.



**Figure 3.** Perspective drawings of tripeptides (side-chains removed) in a conformation centred on a highly populated region of the  $\phi/\psi$  potential energy plots for peptides in a random coil conformation. (a) All residues in L-configuration. (b) Residue 1 in D-configuration and residues 2 and 3 in L configuration. Dihedral angles are  $\phi < 150$ ,  $\psi + 150^\circ$  for L residues and  $\phi + 150$ ,  $\psi - 150^\circ$  for the  $\phi$  residues. The possibility of interactions between side-chains attached to the  $\beta$ -carbons in the 1- and 3-positions is clearly greater in a than in b

**Side-chain Shielding Interactions.**—Further evidence for the lack of a dominant helical contribution to the conformation of these ACTH peptides in aqueous solution comes from a consideration of the chemical shifts of the Phe aromatic protons. We have already noted the increased shielding of some of these protons in the L-Phe ACTH analogues compared with those in either D-Phe ACTH or peptides containing Phe as the only aromatic residue. These shielding differences can readily be explained on the assumption of a random coil conformation of the peptides, and arise from the shielding due to the ring current effect<sup>26</sup> of the Trp-9 indole ring. Figure 3 shows the structures for two tripeptides (such as L-Phe-L-Arg-L-Trp and D-Phe-L-Arg-L-Trp) with  $\phi$  and  $\psi$  dihedral angles set to angles in the region of the  $\phi, \psi$  potential map which would be most heavily populated in a random coil

mixture. The angles chosen are not critical and the conclusions are qualitatively the same for any set of angles chosen in the general region  $\phi - 150$  to  $-90$  and  $\psi + 90$  to  $+15^\circ$  (for L residues).<sup>27</sup> It can be seen in Figure 3 that in the case of L-Phe-L-Arg-L-Trp the two aromatic side-chain residues are on the same side of the peptide backbone whilst this is not the case for the D-Phe isomer. Consideration of this structure, and the rotamer populations of the Phe and Trp side-chains indicates that in some of the conformations two or three of the Phe aromatic protons will be placed 5–7 Å above the plane of the Trp indole ring and result in the observed shielding effects. Thus in a random coil mixture of conformations for a peptide with L-amino-acids there will be substantial populations which can have the side-chains of alternate residues ( $n$  and  $n + 2$ ) close together.

In the comparable structures for a helical conformation the Phe and Trp aromatic rings are well separated and would not result in ring current shifts. The similarity of the aromatic regions of the  $^1\text{H}$  spectra of L-Phe- and D-Phe-ACTH(1–10) in trifluoroethanol suggests that the ring current shielding interactions from the Trp indole ring to the Phe protons seen in aqueous solution are no longer present. This is consistent with the populations of ACTH(1–10) conformations being displaced from the random coil mixture of conformations.

**Conclusions.**—These results indicate that ACTH(1–10) does not form a dominant helical conformation in either aqueous or trifluoroethanol solution. In aqueous solution the large  $J_{\text{NC}}$  coupling constants, large temperature coefficients of NH chemical shifts and the observed shielding interactions involving the alternate residues Trp and Phe indicate that the structure is a random coil. This is further supported by the constancy of the CH  $^1\text{H}$  chemical shifts observed with increasing chain length of the peptides. In trifluoroethanol the conformation has been displaced away from the random coil mixture of conformations as evidenced by the smaller  $J_{\text{NC}}$  coupling constants, the lower values of the NH chemical shift temperature coefficients, and the removal of the Trp–Phe side-chain shielding interactions. The lower temperature coefficients of the chemical shifts of two of the NH protons suggest some contributions from intramolecular hydrogen bonding in trifluoroethanol. While these results are consistent with contributions to the overall conformation from helical structure, these structures are not dominant.

## References

- 1 J. Ramachandran and C. H. Li, *Adv. Enzymol.*, 1967, **29**, 391.
- 2 H. M. Greven and D. de Wied in 'Hormones and the Brain,' eds. D. de Wied and P. A. van Keep, MTP Press, 1980, p. 115.
- 3 A. Witter, H. M. Greven, and D. de Wied, *J. Pharm. Exp. Therap.*, 1975, **193**, 853.
- 4 L. Kisfaludy, M. Löw, T. Szirtes, I. Schön, M. Sarközi, S. Bajusz, A. Turam, A. Jahasz, R. Beke, L. Gráf, and K. Medzih-radszky, 'Chemistry and Biology of Peptides,' ed. J. Meien-hoffer, Univ. of Michigan Press, Ann Arbor, 1972, pp. 299—303.
- 5 M. Löw, L. Kisfaludy, and S. Fermandjian, *Acta Biochem. Biophys. Acad. Sci. Hung.*, 1975, **10**, 229.
- 6 P. Y. Chou and G. D. Fasman, *Biochemistry*, 1974, **13**, 222.
- 7 D. Greef, F. Toma, S. Fermandjian, M. Löw, and K. Kisfaludy, *Biochim. Biophys. Acta*, 1976, **439**, 219.
- 8 F. Toma, S. Fermandjian, M. Löw, and L. Kisfaludy, *Biopolymers*, 1981, **20**, 901.
- 9 D. J. Patel, *Macromolecules*, 1971, **4**, 251.
- 10 R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, 1961, **44**, 1991.
- 11 B. J. Kimber, K. Roth, and J. Feeney, *Anal. Chem.*, 1981, **53**, 1026.
- 12 K. Wüthrich, 'N.M.R. in Biological Research: Peptides and Proteins,' North Holland/American Elsevier, 1976, ch. 2.
- 13 W. A. Gibbons, G. Nemethy, A. Stern, and L. C. Craig, *Proc. Natl. Acad. Sci. USA*, 1970, **67**, 239.
- 14 J. Feeney, G. C. K. Roberts, J. P. Brown, A. S. V. Burgen, and H. Gregory, *J. Chem. Soc., Perkin Trans. 2*, 1972, 601.
- 15 R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, 1962, **5**, 513.
- 16 V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, 1969, **25**, 493.
- 17 P. N. Lewis, F. A. Momany, and H. A. Scheraga, *Isr. J. Chem.*, 1973, **11**, 121.
- 18 R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, 1966, **45**, 2091.
- 19 D. W. Urry, L. Masotti, and J. R. Krivacic, *Biochim. Biophys. Acta*, 1971, **241**, 600.
- 20 M. Ohnishi and D. W. Urry, *Biochim. Biophys. Res. Commun.*, 1969, **36**, 194.
- 21 K. G. R. Pachler, *Spectrochim. Acta*, 1964, **20**, 581.
- 22 K. D. Kopple, G. R. Wiley, and R. Taube, *Biopolymers*, 1973, **12**, 627.
- 23 J. Feeney, *J. Magn. Reson.*, 1976, **21**, 473.
- 24 V. F. Bystrov, *Prog. NMR Spectrosc.*, 1976, **10**, 41.
- 25 P. E. Hansen, J. Feeney, and G. C. K. Roberts, *J. Magn. Reson.*, 1975, **17**, 249.
- 26 C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, 1958, **29**, 1012.
- 27 IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, 1970, **9**, 3471.

Received 19 March 1982; Paper 2/471